# Title: Department of Defense Comments on NTP Toxicological Review of o-nitrotoluene, dated October 2007

Executive Summary - The Draft Background for o-Nitrotoluene provides a thorough and accurate review of the toxicity data for o-nitrotoluene. The review supports the recommendation for listing o-Nitrotoluene (Part B – Recommendation for listing status for o-Nitrotoluene in the RoC) as "*reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals" as described in the NTP "Strength of Evidence Classification System". However, there are weaknesses in the extrapolation of carcinogenicity in animals to humans as discussed more thoroughly below.

## Major Issues –

- The studies reviewed in the background document provide sufficient evidence for carcinogenicity in experimental animals (rats and mice); however, there is insufficient evidence of carcinogenicity in humans based on the available epidemiological studies (3 studies, none specific to o-nitrotoluene exposure). Additionally, the relevance of the animal studies to humans remains uncertain. Although the background document states that "the pathways of metabolism identified in rodents are expected to occur in humans as well," no data to support this statement were provided in the document. Although the two metabolites found in humans following potential exposure to o-nitrotoluene are also produced in rodents, the metabolites found in humans are not the metabolites reported to be the active/carcinogenic metabolite in rodents. Further, because the bioactivation, and in turn the carcinogenicity, of o-nitrotoluene is sex-specific and species-specific (as indicated by differences between metabolite profiles for rats and mice), metabolism in humans can not be assumed to be similar to either sex or any one species of rodent. It remains unclear whether humans metabolize o-nitrotoluene to an active/carcinogenic metabolite.

  Uncertainty exists regarding the mode of action of o-nitrotoluene. The Background document indicates that, based on the bioactivation process demonstrated in rats, females should be resistant to the hepatocarcinogenic effects of o-nitrotoluene because they produce less of the active/carcinogenic metabolite than do males. Females did, however, develop liver adenomas in the NTP study. In the Background document this was cited as evidence that other activation pathways exist. Female rats do produce the active metabolite, although to a lesser extent than

males. Thus, females may be expected to develop liver cancer at a lower rate than males, as was the case in the NTP study. Thus, the development of liver adenomas at high doses in the females does not necessarily indicate that other activation pathways exist.

The development of tumors in various tissues other than the liver may, however, indicate that o-nitrotoluene may have several modes of action. Additionally, gene expression studies indicate that the hemangiosarcomas in mice may be due to the genotoxic effects of o-nitrotoluene. In contrast, the genotoxicity studies conducted (micronucleus) with the same species indicate that o-nitrotoluene is not genotoxic. Uncertainties regarding the mode(s) of action of o-nitrotoluene were only briefly addressed in the Background document.

The uncertainties regarding the mode of action of o-nitrotoluene and the applicability of the animal data to humans (given the specific metabolic activation pathway necessary to produce liver tumors) represent significant data gaps. Given the requirement for *in vivo* metabolism to produce an active/carcinogenic compound, it would be appropriate to conduct *in vivo* genotoxicity studies with the various target tissues identified in the NTP bioassay to help elucidate the mode(s) of action. Metabolism studies in species more similar to humans may help determine whether the metabolic activation pathway identified in rats also occurs in humans and would clarify the applicability of the rodent data to humans.

Substantive Comments - Several statements about Dinitrotoluene provided in the “Other Relevant Data” section are incorrect and should be corrected.

Statements indicating that the metabolism in humans is similar to that of rodents are not supported (see discussion above).

- See table.

Editorial Comments - See table.

| Department of Defense Comments on the<br>National Toxicology Program (NTP) Report on Carcinogens Listing ortho-Nitrotoluene | | |
|---|---|---|
| Comments submitted by: Office of the Secretary of Defense, Emerging Contaminants Directorate | Organization: U.S. Department of Defense | Date Submitted: 24 April 2008 |

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

| Comment No. | Section | Page & Paragraph (enter "Global" if report section-wide) | Comment | Suggested Action, Revision and References (if necessary) | Category* |
|---|---|---|---|---|---|
| 1 | Executive Summary: ADME | Pg. vii<br>Para1,<br>Line1 | The statement, "Metabolites of o-nitrotoluene have been detected in the urine of factory workers ..." does not indicate which metabolites have been found. | Suggest adding that o-nitrobenzoic acid and o-nitrobenzyl alcohol have been found in urine of factory workers. The workers were potentially exposed during the production of dinitrotoluenes and trinitrotoluene; however, no significant correlation was found between o-nitrotoluene concentrations in the air and urinary metabolites. | S |
| 2 | Executive Summary: ADME | Pg. vii<br>Para 2<br>Line 13 | Pertinent information missing. | Suggest indicating that the o-aminobenzyl alcohol that was found only in rats is the metabolite that is subsequently converted to o-aminobenzyl sulfate, which is the proposed proximal carcinogenic metabolite of o-nitrotoluene. | S |
| 3 | Executive Summary: ADME | Pg. vii<br>Para 2<br>Line15-18 | Pertinent information missing. | Suggest indicating that the differences in biliary metabolism/excretion between male and female rats are reported to account for the differences in carcinogenicity of o-nitrotoluene between males and females. | S |
| 4 | Studies of Cancer in Experimental Animals | Pg. 21<br>Para. 4<br>Line 28 | Doses given only as ppm concentrations in feed. | Suggest indicating that the authors calculated these doses to be approximately 40-700 mg/kg/day based on feed consumption. The actual dose received by each animal, however, may have been considerably variable due to group housing (5 per cage) of animals. | S |

| Department of Defense Comments on the<br>National Toxicology Program (NTP) Report on Carcinogens Listing ortho-Nitrotoluene | | |
|---|---|---|
| Comments submitted by: Office of the Secretary of Defense, Emerging Contaminants Directorate | Organization: U.S. Department of Defense | Date Submitted: 24 April 2008 |

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

| Comment No. | Section | Page & Paragraph (enter "Global" if report section-wide) | Comment | Suggested Action, Revision and References (if necessary) | Category* |
|---|---|---|---|---|---|
| 5 | Studies of Cancer in Experimental Animals | Pg. 26<br>Para. 2<br>Line 19-20 | No indication of how average daily dose was determined. | Suggest indicating that average daily doses were calculated based on feed consumption for group housed animals (3-5 rats/cage) and individual doses may have varied considerably. | S |
| 6 | Studies of Cancer in Experimental Animals | Pg. 31<br>Para 2<br>Line 9 | Doses given only as ppm concentrations in feed | Suggest indicating that the authors calculated these doses to be approximately 100-1700 mg/kg/day based on feed consumption. The actual dose received by each animal, however, may have been considerably variable due to group housing (5 per cage) of animals. | S |
| 7 | Other Relevant Data | Pg. 71<br>Para 2<br>Line 8-9 | The text incorrectly states that 2,4-dinitrotoluene has initiating and promoting activity, whereas 2,6-dinitrotoluene had only promoting activity. | Revise statement to say that 2,6-dinitrotoluene has initiating and promoting activity, whereas 2,4-dinitrotoluene has only weak promoting activity (Popp and Leonard, 1982, Toxicologic Pathology 10 (2): 190-196). | S |
| 8 | Other Relevant Data | Pg. 71<br>Para 2<br>Line 10-11 | Text incorrectly cites Leonard et al (1987) as noting that 2,4-dinitrotoluene was weakly hepatocarcinogenic. Leonard et al. (1987) states that 2,4-dinitrotoluene is not hepatocarcinogenic. | Revise text to correctly reflect the statements in Leonard et al. (1987) | S |

| Department of Defense Comments on the<br>National Toxicology Program (NTP) Report on Carcinogens Listing ortho-Nitrotoluene | | |
|---|---|---|
| Comments submitted by: Office of the Secretary of Defense, Emerging Contaminants Directorate | Organization: U.S. Department of Defense | Date Submitted: 24 April 2008 |

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

| Comment No. | Section | Page & Paragraph (enter "Global" if report section-wide) | Comment | Suggested Action, Revision and References (if necessary) | Category* |
|---|---|---|---|---|---|
| 9 | Other Relevant Data | Pg. 71<br>Para 2<br>Line 12-14 | Text incorrectly states that technical grade dinitrotoluene contains 5 to 10 times more of the 2,6-isomer than the 2,4-isomer. | Revise text to reflect that technical grade dinitrotoluene has more of the 2,4-isomer than the 2,6-isomer (approximately 76.5% 2,4-dinitrotoluene and 19% 2,6-dinitrotoluene). I believe the statement is intended to reflect the differences in doses of the two isomers in the NCI and CIIT studies. If that is the case, the text should be edited to state that due to the differences in composition of the DNT solution used in these studies (NCI=5% 2,6-DNT and CIIT=19% 2,6-DNT) and the dosing rate (NCI=5.6 and 14.0 mg/kg/d and CIIT=3.5, 14, or 35 mg/kg/day), the total dose of 2,6-DNT was 5-10 greater in the CIIT study than in the NCI study. | S |
| 10 | Part B – Rec. for Listing o-nitrotoluene | Other Relevant Data (Para. 1) | Text states that the pathways of metabolism identified in rodents are expected to occur in humans. This statement is not supported by the Background document which states that 2 metabolites have been found in humans. The metabolites found in humans are not the metabolites reported in rodents as being the active/carcinogenic metabolite. Further, due to the differences in carcinogenicity between sexes as a result of differences in metabolism and biliary excretion, one can not make a generalized statement that the metabolism in humans is expected to be similar to rodents. | Suggest removing the statement or provide sufficient evidence in the background document to support this claim. | S |

| Department of Defense Comments on the<br>National Toxicology Program (NTP) Report on Carcinogens Listing ortho-Nitrotoluene | | |
|---|---|---|
| Comments submitted by: Office of the Secretary of Defense, Emerging Contaminants Directorate | Organization: U.S. Department of Defense | Date Submitted: 24 April 2008 |

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

| Comment No. | Section | Page & Paragraph (enter "Global" if report section-wide) | Comment | Suggested Action, Revision and References (if necessary) | Category* |
|---|---|---|---|---|---|
| 11 | Part B – Rec. for Listing o-nitrotoluene | Other Relevant Data (Para 3) | The text states that o-nitrotoluene was shown to induce chromosomal aberrations in Chinese Hamster Lung (CHL) cells and human peripheral lymphocytes and that it induces micronuclei in CHL cell lines. These statements do not match what is reported in the Background document which does not indicate that any studies have been done with CHL cell lines. | Suggest removing these statements or provide sufficient evidence (and references) in the background document to support these statements. | S |
| 12 | Introduction | Pg. 1<br>Para. 1 | States "10 million to 50 million pounds per year" which is in conflict with Page V, Line 18 that states, "10 million to 50 million pounds for every four-year period". | Clarification needed. | E |